

User Manual / Handbook

RT² Profiler™ PCR Array System

Pathway-Focused Gene Expression Profiling
Using Real-Time PCR

See Purchaser Notification for limited use license and warranty information (page 41).

Part #1022A Version 5.01 9/16/2010

RT² Profiler PCR Array System

Pathway-Focused Gene Expression Profiling Using Real-Time PCR

User Manual

(For Catalog Numbers Prefixed by: PAHS, PAMM, PARN, PADM, and PAQQ)

QIAGEN Catalog # 330231, 330111, 330131

Ordering and Technical Service Contact Information:

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- Fax: 1-888-465-9859 (US) 301-682-7300 (outside US)
- On-line Order: www.SABiosciences.com
- E-MAIL: order@SABiosciences.com (to place an order)
support@SABiosciences.com (for technical support)

You may place orders by fax, e-mail or from our website. Each order should include the following information:

- Your contact information (name, phone, email address)
- Product name, catalog number and quantity
- Purchase order number or credit card information (Visa or MasterCard)
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For more information, visit us at www.SABiosciences.com

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I. Background and Introduction

Real-time reverse transcription (RT) PCR is the most sensitive and reliable method for gene expression analysis. Its wide dynamic range makes real-time RT-PCR the preferred choice for the simultaneous quantification of both rare and abundant genes in the same sample. The RT² Profiler PCR Array takes advantage of real-time PCR performance and combines it with the ability of microarrays to detect the expression of many genes simultaneously. RT² Profiler PCR Arrays are designed to analyze a panel of genes related to a disease state or biological pathway. The product is especially suitable for researchers who are more familiar with or prefer real-time PCR technology but are looking for the multi-gene profiling capabilities of a microarray.

To complete the PCR Array procedure, start by converting your experimental RNA samples into first strand cDNA, the template for the polymerase chain reaction, using our RT² First Strand Kit. (See Figure 1 for an overview of the PCR Array procedure.) Then, mix your template with one of our instrument-specific and ready-to-use RT² qPCR Master Mixes. Aliquot the mixture into each well of the same plate containing pre-dispensed gene-specific primer sets. Perform PCR, and finally, determine relative expression with your real-time instrument and the $\Delta\Delta C_t$ method.

Each array contains a panel of 96 or 384 primer sets for a thoroughly researched set of 84 or 370 relevant, pathway- or disease-focused genes, plus five housekeeping genes and three RNA and PCR quality controls. The 96-gene PCR Arrays are available in a 96-well plate format, a 100-well Rotor-Disc format, and a 384-well plate format, containing either one or four replicates, respectively, of the 96-primer set panel. (See Figure 2 for the layout of typical PCR Arrays.) The PCR Array 384HT products (catalog numbers greater than 3000) contain a 370-gene panel in a 384-well plate format. SABiosciences's qPCR Assays, Master Mixes, and first strand kit have been optimized hand-in-hand for SYBR[®] Green real-time RT-PCR detection, providing the PCR Arrays with superior sensitivity and wide linear dynamic ranges. The simplicity of the PCR Arrays also makes them accessible for routine use in every research laboratory.

Benefits of the RT² Profiler PCR Arrays:

- ❖ **Pathway Focused:** Profile the expression of a panel of genes relevant to a pathway or disease state.
- ❖ **Simple and Accurate:** Simple real-time PCR procedure provides high sensitivity and wide dynamic range.
- ❖ **Designed for Routine Use:** Bring expression profiling to any lab with a real-time PCR instrument.

Combine microarray profiling capabilities with real-time PCR performance!

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How It Works

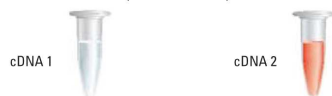
Isolate RNA from your Experimental Samples.

Start with as little as 25 ng of total RNA
(1 µg is recommended, treat with DNase)



Prepare cDNAs from your RNA Samples.

Use the RT⁺ First Strand Kit (Cat. No. C-03)



Add cDNA to RT⁺ qPCR Master Mix.

RT⁺ qPCR Master Mixes contain SYBR Green and reference dye.



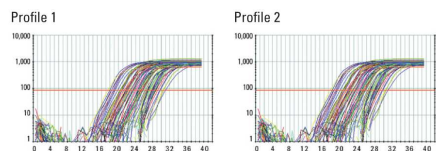
Aliquot the Mixture Across Your PCR Arrays.

Each PCR Array profiles the expression of 84 pathway-specific genes plus controls.



Perform Thermal Cycling

Collect real-time amplification data using your instrument's software.



Analyze Changes in Gene Expression.

Analysis is straightforward. A simple cut-and-paste operation puts the C_t values collected by your real-time instrument into an analysis spreadsheet. Fold changes in gene expression between your samples are calculated automatically.

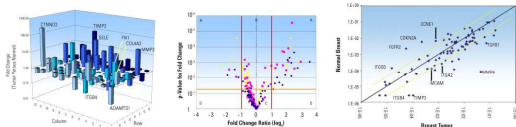
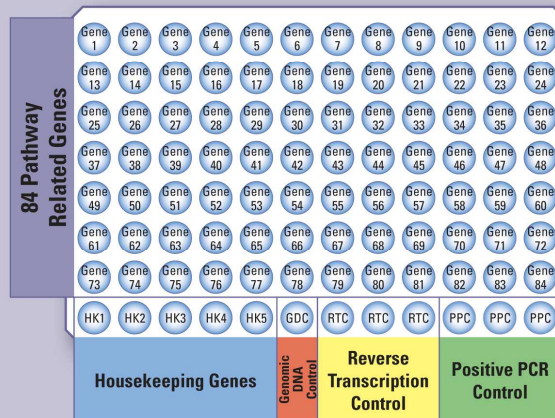


Figure 1: Overview of the PCR Array Procedure

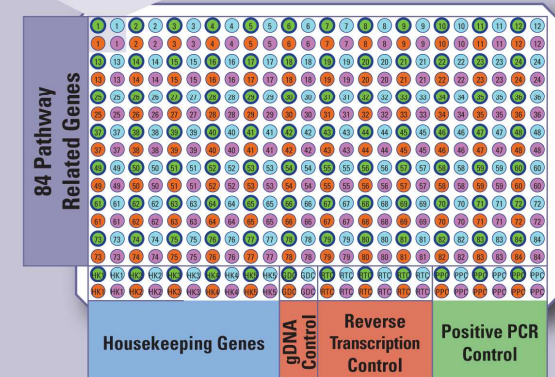
2A: Standard PCR Array Layouts

96-well PCR Array



Sample Loading Order
A B
C D

384-well PCR Array (4x96)



2B: PCR Array 384 HT Layout

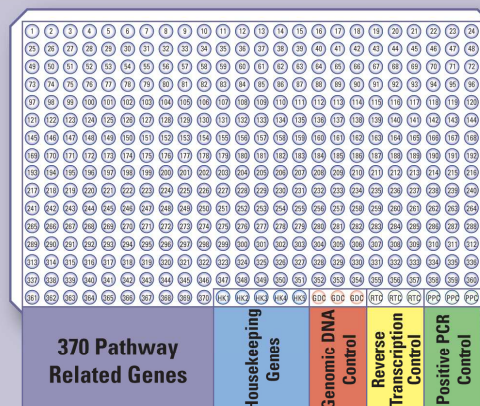


Figure 2: Layout of the Cataloged Pathway-Focused PCR Arrays



Figure 2C. Layout of the Cataloged Pathway-Focused Rotor-Gene Q Rotor-Disc 100 PCR Array

Figure 2A: Layout of the 96-Well and 384-Well PCR Arrays

Wells A1 through G12 contain a real-time PCR assay for genes from the same biological pathway or the same disease state or genes that are otherwise functionally-related.

The product information included with each cataloged PCR Array contains a list of the pathway-focused and housekeeping genes on the array.

Housekeeping Assay Panel: Wells H1 through H5 contain a housekeeping gene panel to normalize PCR Array data.

Genomic DNA Controls (GDC): Well H6 contains the Genomic DNA Control (GDC).

Reverse Transcription Controls (RTC): Wells H7 through H9 contain replicate Reverse Transcription Controls (RTC).

Positive PCR Controls (PPC): Wells H10 through H12 contain replicate Positive PCR Controls (PPC).

The 384-well (4 x 96) format of the PCR Arrays includes four replicates of the same 96-well format, in which each two-by-two set of wells (wells labeled 1 - 4 in gray above) contains the same primer set represented by the 96-well designations.

Figure 2B: Layout of the PCR Array 384HT

Wells A1 through P10 (1-370) each contain a real-time PCR assay for genes from the same biological pathway or the same disease state or genes that are otherwise functionally-related.

The product information included with each cataloged PCR Array contains a list of the pathway-focused and housekeeping genes on the array.

Housekeeping Assay Panel: Wells P12 through P15 contain a housekeeping gene panel to normalize PCR Array data.

Genomic DNA Controls (GDC): Wells P16 through P18 contain replicate Genomic DNA Controls (GDC).

Reverse Transcription Controls (RTC): Wells P19 through P21 contain replicate Reverse Transcription Controls (RTC).

Positive PCR Controls (PPC): Wells P22 through P24 contain replicate Positive PCR Controls (PPC).

Figure 2C: Layout of the 100-Well Rotor-Gene Q Rotor-Disc 100 PCR Array

The 96 real-time assays found in the 96-well PCR plate-based arrays are located in wells 1 through 96 of the Rotor-Gene Q ring (plate A1-A12 → Ring 1-12, plate B1-B12 → Ring 13-24, etc.). To maintain data analysis compatibility, wells 97-100 do not contain real-time assays but will contain master mix to account for weight balance.

RT² Profiler PCR Array Controls - Definitions

The Genomic DNA Control (GDC) is an assay that specifically detects non-transcribed genomic DNA contamination with a high level of sensitivity. The Reverse Transcription Control (RTC) tests the efficiency of the RT² First Strand Kit (C-03) reaction with a primer set detecting the template synthesized from the kit's built-in external RNA control. The Positive PCR Control (PPC) tests the efficiency of the polymerase chain reaction itself using a pre-dispensed artificial DNA sequence and the primer set that detects it.

The sets of replicate control wells (GDC, RTC, & PPC) also test for inter-well, intra-plate consistency.

Custom PCR Arrays have your specified layout, and the product information literature enclosed with the array reiterates that layout and the genes included.

II. Materials Provided:

The PCR Arrays are available in 8 different plate formats, each tailored to a specific subset of real-time PCR instruments & associated blocks. Formats A, C, D, and F are 96-well plates, Formats E & G are 384-well plates, Format R are 100-well discs, and Format H is 96 x 96 Chip compatible.

Format	For Real-Time Instruments	Plate
A	ABI “ standard ” blocks: 5700, 7000, 7300, 7500, 7700, 7900HT, ViiA™ 7 (96-well block) Bio-Rad: iCycler®, iQ5, MyiQ, MyiQ2, Chromo4 (MJ Research) Eppendorf: MasterCycler® ep RealPlex® 2, 2s, 4, 4s Stratagene: Mx3005p®, Mx3000p® Takara: TP-800	96-well
C	ABI: 7500 FAST block, 7900HT FAST block, StepOnePlus™, ViiA™ 7 FAST block	96-well
D	Bio-Rad: CFX96™, Opticon® and Opticon 2 (MJ Research) Stratagene: Mx4000®	96-well
E	ABI: 7900HT (384-well block), ViiA™ 7 (384-well block) Bio-Rad: CFX384™	384-well
F	Roche: LightCycler 480 96-well block	96-well
G	Roche: LightCycler 480 384-well block	384-well
H	Fluidigm BioMark	96x96 Chip
R	QIAGEN Rotor-Gene Q	100-well Rotor-Disc 100

NOTE: The format of the PCR Array is indicated by the last letter of the catalog number. Be sure that you have the correct PCR Array format for your instrument before starting the experiment.

The 96-well Plate and 100-well Disc PCR Arrays (Formats A, C, D, F, and R) are shipped in sets of 2, 12, or 24, while the 384-well PCR Arrays (Formats E and G) are shipped in sets of 4. The PCR Array 384HT is shipped in sets of 2, 12, or 24. The PCR Array Plate Format H is shipped in a Format A plate in solution (on ice).

Each PCR Array shipment includes the arrays and either 12 optical thin-wall 8-cap strips (Formats A & D), 1 optical adhesive film (Formats C, E, F, and G), or 1 Rotor-Disc heat sealing film (Format R) per array.

Each 96x4 Format 384-Well PCR Array (Formats E & G) also includes one set of 4 384EZLoad™ Covers (Catalog #PA-384) for each PCR Array provided in the package.

NOTE: Each 384EZLoad™ Cover is for a Single Use ONLY.

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Shipping Conditions:

- PCR Arrays in formats A, C, D, E, F, G, and R are shipped at Room Temperature (RT), on Dry Ice (DI), or Blue Ice (BI) depending on destination & accompanying products.
- PCR Arrays in format H are shipped on dry ice or blue ice packs.

Storage Conditions: Keep plates and system components at -20°C for long-term storage. When stored properly at -20°C, their quality is guaranteed for 6 months.

III. Additional Materials Required:

- A. **RNA Isolation:** See Page 15 for specific recommendations.
- B. High-quality, nuclease-free H₂O. **Do NOT USE DEPC H₂O.**
- C. **RT² First Strand Kit** (Cat. No. C-03/330401)
MANDATORY for a Complete and Successful Experiment
For Reverse Transcription Control Detection (RTC, Wells H7 through H9)
- D. **SABiosciences RT² qPCR Master Mix**
MANDATORY for a Complete and Successful Experiment
 Be sure to pick the correct one for the instrumentation in your laboratory.

1. 96-Well & 384-Well (96 x 4 Format) Block PCR Arrays

RT² SYBR Green / ROX qPCR Master Mix: Specifically designed for:

- ABI 5700, 7000, 7300, 7500 (Standard & FAST), 7700, 7900HT 96-well block (Standard & FAST) and 384-well block, StepOnePlus
- Eppendorf Mastercycler ep realplex 2/2S/4/4S
- Stratagene Mx3000p, Mx3005p, Mx4000
- TaKaRa TP-800

Catalog Number

Size

PA-012/ 330520	For 2 96-well RT ² Profiler PCR Arrays
PA-012-12/ 330522	For 12 96-well RT ² Profiler PCR Arrays
PA-012-24/ 330523	For 24 96-well RT ² Profiler PCR Arrays
PA-012-8/ 330521	For 4 384-well RT ² Profiler PCR Arrays

RT² SYBR Green / Fluorescein qPCR Master Mix: Specifically designed for:

- Bio-Rad iCycler, iQ5, MyiQ, MyiQ2

Catalog Number

Size

PA-011/ 330510	For 2 96-well RT ² Profiler PCR Arrays
PA-011-12/ 330512	For 12 96-well RT ² Profiler PCR Arrays
PA-011-24/ 330513	For 24 96-well RT ² Profiler PCR Arrays
PA-011-8/ 330511	For 4 384-well RT ² Profiler PCR Arrays

RT² SYBR Green qPCR Master Mix: Specifically designed for instrumentation that does not require a reference dye:

- Bio-Rad CFX96, CFX384, Chromo4, Opticon 2
- Roche LightCycler 480 (96-well & 384-well)
- All Others

Catalog Number

Size

PA-010/ 330500	For 2 96-well RT ² Profiler PCR Arrays
PA-010-12/ 330502	For 12 96-well RT ² Profiler PCR Arrays
PA-010-24/ 330503	For 24 96-well RT ² Profiler PCR Arrays
PA-010-8/ 330501	For 4 384-well RT ² Profiler PCR Arrays

2. 100-Well Ring PCR Array for QIAGEN Rotor-Gene Q 100 ("R" Plate Format: Rotor-Disc 100)

RT² FAST SYBR Green / ROX qPCR Master Mix: Specifically designed for:

- QIAGEN Rotor-Gene Q

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Catalog Number	Size
PA-042/ 330620	For 2 100-well RT ² Profiler PCR Arrays
PA-042-12/ 330622	For 12 100-well RT ² Profiler PCR Arrays
PA-042-24/ 330623	For 24 100-well RT ² Profiler PCR Arrays

3. PCR Array 384HT

The PCR Array 384HT two (2), twelve (12), and twenty-four (24) packs (Formats E & G) require a quantity of two (2) of the correct master mixes for your instrument of the size for the corresponding 96-well PCR Array packs (2 X PA-01# or 2 X PA-01#-12 or 2 X PA-01#-24).

4. Custom PCR Arrays: 330131

Custom PCR Arrays are available in a number of formats, allowing the customer to choose the number of genes to study and the number of sample replicates that may be needed. Please select a master mix package size that provides sufficient volume for your samples.

E. Equipment:

1. For recommendations on specific real-time instrumentation (thermal cyclers with fluorescent detection), see the list of master mixes and plate formats above.

NOTE: *The PCR Arrays can only be used in 96-well and 384-well real-time PCR instruments. PCR Arrays can not be used in the Cepheid SmartCycler® or the Roche LightCycler 2.0.*

2. Calibrated Multi-Channel Pipettor
3. RNase / DNase-free pipette tips and tubes

IV. Complementary Products:

A. RT² RNA QC PCR Array (Optional):

Pick the correct catalog number for your species of interest (see below) and the correct plate format for the instrument in your lab. (See table on Page 8.)

<u>Human</u> RT ² RNA QC PCR Array	Cat. No. PAHS-999 (330291)
<u>Mouse</u> RT ² RNA QC PCR Array	Cat. No. PAMM-999 (330291)
<u>Rat</u> RT ² RNA QC PCR Array	Cat. No. PARN-999 (330291)

B. XpressRef™ Universal Total RNA:

Universal RNA to control PCR conditions is available from the following species:

Human XpressRef™ Universal Total RNA	Cat. No. GA-004 / 338112
Mouse XpressRef Universal Total RNA	Cat. No. GA-005 / 338114
Rat XpressRef Universal Total RNA	Cat. No. GA-006 / 338116

C. FFPE/Paraffin Block Applications

- a. RNA Extraction Kit: PA-023/330471
- b. FFPE PreAMP cDNA Synthesis Kit: C-07/330461
- c. FFPE PreAMP Primer Mixes: PFX-####/330151

D. RT² PCR Array Loading Reservoir: PA-027/338162

V. Protocol:

Please read through this entire protocol before beginning your experiment.

RNA samples are very sensitive to RNase digestion; therefore, wear gloves and maintain an RNase-free work area while performing this protocol.

NOTE: Master Mix and First Strand Synthesis Considerations

The performance of our RT² Profiler PCR Arrays is only guaranteed with SABiosciences RT² qPCR Master Mixes and the RT² First Strand Kit. Therefore, the use of the complete RT² Profiler PCR Array System is absolutely essential for obtaining accurate real-time PCR profiling results.

The chemically-modified and tightly controlled HotStart enzyme and other proprietary chemical components in our RT² qPCR Master Mixes uniquely provide more accurate SYBR Green results by preventing the amplification of primer dimers and other non-specific products. They also help ensure high amplification efficiencies even for those genes that are the most difficult to amplify. When we test other sources of enzymes with our PCR Arrays, we frequently see primer dimers and other non-specific products that confound SYBR Green-based real-time PCR detection. Because each instrument uses a different reference dye to normalize their optics, be sure that you use the correct master mix for the instrumentation in your laboratory.

The RT² First Strand Kit includes a proprietary buffer to eliminate any residual genomic DNA contamination in your RNA samples before it can be amplified into secondary products that would otherwise cause false positive signals. The Reverse Transcription Controls (RTC) on the PCR Array can only be evaluated with the built-in external RNA control of the RT² First Strand Kit. These controls do not yield results when used with other sources of reverse transcriptases or first strand synthesis kits. The buffer components and the magnesium concentration in our RT² First Strand Kit are also more compatible with our PCR master mixes than other enzymes or kits providing the PCR Arrays with maximum levels of sensitivity with ng to µg amounts of total RNA.

NOTE: Preparing a Workspace Free of DNA Contamination

For accurate and reproducible PCR Array results, it is very important to avoid contamination of the assay with foreign DNA. Any DNA contamination will artificially inflate the SYBR Green signal yielding skewed gene expression profiles and false positive signals. The most common sources of DNA contamination are the products of previous experiments spread into the air of your working environment. Please follow the recommendations below on how to set up and maintain a working environment free of DNA contamination.

1. Wear gloves throughout the procedure. Use only fresh PCR-grade reagents (H₂O) and lab ware (tips and tubes).
2. Physically separate the workspaces used for PCR setup and post-PCR processing or non-PCR operations. Decontaminate your PCR workspace and lab ware (pipettor barrels, tube racks, etc.) before each new use with UV light to render any contaminating DNA ineffective in PCR through the formation of thymidine dimers or with 10% bleach to chemically inactivate and degrade any DNA.
3. Close all tubes containing PCR products once you are finished adding or removing volumes. Before discarding any lab ware (tips or tubes) containing PCR products or other DNA, treat with 10% bleach.
4. Do not remove the PCR Array plate from its protective sealed bag until immediately ready to use. Do not leave lab ware (tubes and tip boxes) exposed to the air for long periods of time.
5. Do not open any previously run and stored PCR Array plate. Removing the thin-wall 8-cap strips or the adhesive film from PCR Arrays releases PCR product DNA into the air where it will contaminate and confound the results of future real-time PCR experiments.

A. RNA Preparation and Quality Control:

High quality RNA is ESSENTIAL for obtaining good real-time PCR results.

The most important prerequisite for any gene expression analysis experiment is consistent, high-quality RNA from every experimental sample. Therefore, the sample handling and RNA isolation procedures are critical to the success of the experiment. Residual traces of proteins, salts or other contaminants will either degrade the RNA or decrease the efficiency of (if not block completely) the enzyme activities necessary for optimal reverse transcription and real-time PCR performance.

1. Recommended RNA Preparation Methods:

High quality total RNA for your real-time PCR experiment must be prepared using one of the following methods, each specific for your biological sample:

a. Cultured Cells:

Use the Qiagen RNeasy[®] Mini Kit (Catalog # 74104).

- You must perform the recommended on-column DNase treatment step (RNase-Free DNase Set: 79254).

b. Tissue Samples:

- First, extract RNA from the tissue using the QIAzol/TRIzol[®] protocol. Be sure to use a sufficient amount of QIAzol/TRIzol reagent. During homogenization, add a volume of reagent at least ten times greater than the tissue volume.
 - After the ethanol precipitation step, further clean up the RNA using the Qiagen RNeasy Mini Kit (Catalog # 74104).
- You must perform the recommended on-column DNase treatment step (RNase-Free DNase Set: 79254).

c. Formalin-fixed Paraffin-embedded (FFPE) Samples:

- Please refer to protocol for SABiosciences RT² FFPE RNA Extraction Kit (Catalog # PA-023/330471)

d. Small Samples Yielding Less than 100 ng Total RNA:

- Please refer to protocol for Molecular Devices[®] PicoPure[®] RNA Isolation Kit (Catalog # KIT0204)

e. Whole Blood Samples:

- Before RNA preparation, red blood cells (RBC) must be removed from whole blood samples using a density gradient centrifugation medium (for example, Lymphoprep[®], Greiner Bio-One, Catalog # 1031966).
 - The white blood cell fraction is then used for RNA isolation with the Qiagen RNeasy[®] Mini Kit (Catalog # 74104).
- You must perform the recommended on-column DNase treatment step.
 - iii. Alternatively, the PAXgene[™] Blood RNA Kit (Qiagen, Catalog # 762164) can also be used to prepare total RNA from whole blood samples.

f. Total RNA Isolated Using a Phenol-Based Method:

If you have already prepared total RNA from any biological source material using a phenol-based method (such as QIAzol, TRIzol[®], RNeasy, etc.), you must clean up the RNA with the Qiagen RNeasy[®] Mini Kit (Catalog # 74104).

- You must perform the recommended on-column DNase treatment step (RNase-Free DNase Set: 79254).

Technical Support: 888.503.3187 (US) 301.682.9200

g. For Other Biological Samples:

Refer to the existing literature to find isolation protocols for high-quality RNA from other biological samples or contact one of our Technical Support representatives.

For best results from the PCR Array, all RNA samples should be suspended in the RNase-free water provided with the RNA Isolation kit. **DO NOT** use DEPC-treated water!

2. RNA Quality Control:

For best results from the PCR Array, all RNA samples should also demonstrate consistent quality according to the following criteria:

a. RNA Concentration and Purity by UV Spectrophotometry

NOTE: Prepare dilutions and measure absorbance in 10 mM Tris, pH 8.0 buffer. The spectral properties of nucleic acids are highly dependent on pH.

- i) $A_{260}:A_{230}$ ratio should be greater than 1.7.
- ii) $A_{260}:A_{280}$ ratio should be 1.8 to 2.0.
- iii) Concentration by A_{260} should be greater than 40 μg / ml total RNA

b. Ribosomal RNA band integrity

Electrophorese a fraction of each RNA sample on a denaturing agarose gel or on an Agilent BioAnalyzer using an RNA 6000 Nano LabChip® and verify that there is a sharp distinction at the small side of both the 18S and 28S ribosomal RNA (rRNA) bands or peaks. Any smearing or shoulder to the rRNA bands or peaks indicates that degradation has occurred in the RNA sample.

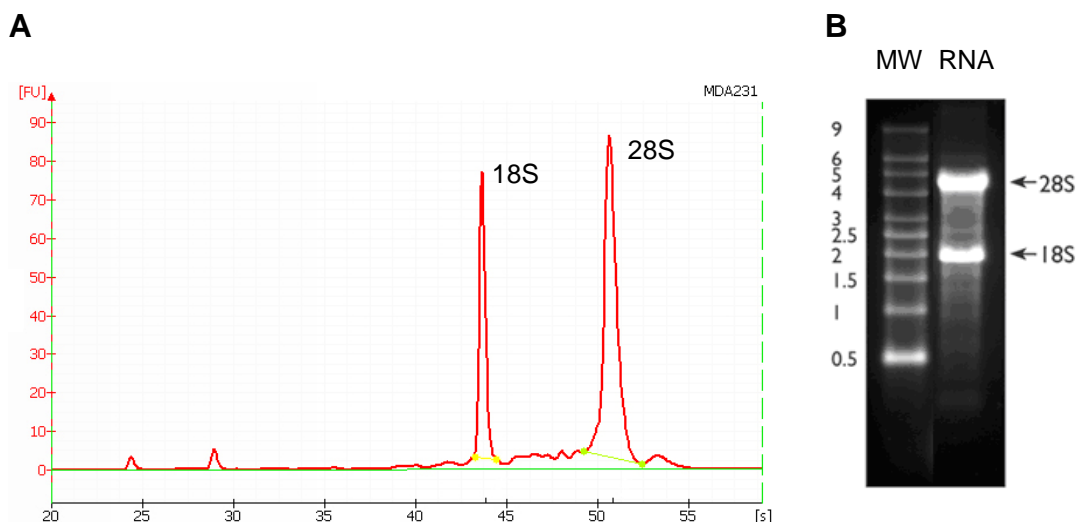


Figure 3: Good Ribosomal RNA Band Integrity Is Important for Optimal PCR Array Results.

Panel A displays an Agilent BioAnalyzer electropherogram of a high-quality total RNA preparation showing sharp peaks without shoulders (especially to the left of each peak) for the 18S and 28S ribosomal RNA (left to right). Panel B, right-hand lane, displays an analysis of the same high-quality total RNA preparation by agarose gel electrophoresis demonstrating sharp bands (especially at the bottom of each band) for the 28S and 18S ribosomal RNA (top to bottom).

Because some contaminants are difficult to detect by simply looking at RNA integrity and can be missed by UV spectrophotometry, it is essential to choose the proper RNA isolation method for your biological sample as described above.

For reliable data from RT² Profiler PCR Arrays & RT² qPCR Primer Assays, a RNA Integrity Number (RIN) of 7 or higher is recommended.

NOTE: Consistent RIN values across multiple samples within each experiment are desirable for reliable quality data comparisons.

NOTE: Samples with degraded RNA, such as from FFPE blocks/slides, with RINs less than 7, **may** be of sufficient quality, but the final PCR Array/ Primer Assay (or gene expression) data must be checked for quality by the research investigator.

c. The RT² RNA QC PCR Array (Optional):

The RT² RNA QC PCR Array and the RT² First Strand Kit (each sold separately) test for a number of RNA quality control parameters including:

- High and low housekeeping gene expression levels
- Reverse transcription and polymerase chain reaction efficiency
- Genomic and general DNA contamination

The RNA QC PCR Arrays are particularly useful for researchers who are unsure of their RNA isolation technique. Follow the recommendations for the use and interpretation of the RT² RNA QC PCR Array found in the RT² RNA QC PCR Array User Manual.

3. Genomic DNA Contamination:

Eliminating genomic DNA contamination is essential for obtaining optimal real-time gene expression profiling results using the PCR Array. The Genomic DNA Control in each PCR Array specifically tests for genomic DNA contamination in each sample during each run. A GDC threshold cycle value less than 35 indicates the presence of a detectable amount of genomic DNA contamination that should be addressed.

We highly recommend performing the on-column DNase treatment step in the Qiagen RNeasy® Mini Kit (Catalog # 74104) followed by using the RT² First Strand Kit (C-03) to remove any and all residual contamination from your RNA samples.

i. Amount Considerations:

The PCR Array System yields results with as little as 25 ng or as much as 5 µg total RNA per array. The **new RT² Nano PreAMP and RT² FFPE PreAMP technologies** allow for gene expression analysis from as little as 1 ng of total RNA (or 100 ng RNA from FFPE samples). Please refer to the corresponding user manuals for additional information.

The optimal amount of starting material depends on the relative abundance of the transcripts of interest. Lower abundance transcripts require more RNA; higher

abundance transcripts require less RNA. Greater amounts of input total RNA yield a greater number of positive calls; that is, genes expressed in the linear dynamic range of the method. Lower amounts of input total RNA yield a smaller number of positive calls and increase false negative calls.

The use of the RT² First Strand Kit (C-03/330401) maximizes the number of positive calls at low amounts (25 ng) of total RNA over other sources of reverse transcriptase and first strand synthesis kits. For successful results and maximum positive call rates, we recommend that first time users try starting with *1.0 µg of total RNA for 96-well plate format, 0.8 µg of total RNA for 100-well Rotor-Disc format, 400 ng of total RNA for 384-well (4 x 96) plate format, and 1.0 µg of total RNA for 384-well HT plate format PCR Array*. It is also important to use a consistent amount of total RNA for all samples in a single experiment to be characterized and compared.

B. RT² First Strand Kit: C-03/330401

NOTE: The use of SABiosciences's RT² First Strand Kit (Cat. No. C-03/330401) is critical for detecting the Reverse Transcription Controls (RTC, Wells H7-H9) and for obtaining the best results from the PCR Array. (See Pages 11 and 15 for more information)

NOTE: RNA samples must meet the standards of integrity and purity from protein, organics, and genomic DNA contamination described on the previous two pages.

NOTE: Use the same amount of total RNA in this reaction for every sample. First time users are recommended to start with 1.0 µg of total RNA for 96-well plate format, 0.8 µg of total RNA for 100-well Rotor-Disc format, 400 ng of total RNA for 384-well (4 x 96) plate format, and 1.0 µg of total RNA for a 384-well HT plate format PCR Array. Lower amounts of total RNA than 100 ng will dramatically increase the false negative rate of the PCR Array method.

NOTE: Scientists using the **Fluidigm BioMark** Real-Time PCR System: Please refer to the specification sheet for the "RT² First Strand Kit – BioMark" (Cat. # C-10/330431) for first-strand cDNA synthesis protocol and for sample/plate preparation instructions.

NOTE: Do not use DEPC treated H₂O. Use high-quality, nuclease-free H₂O. If you are not sure whether your RNase, DNase-free water has been DEPC treated, please check with the supplier.

NOTE: Carefully pipette reagents from reagent tubes, starting with pipette tip at top of tube and working down slowly.

1. Briefly (10-15 seconds) spin down all reagents.
2. Prepare the **Genomic DNA Elimination Mixture**^{**}:
 - a. For each RNA sample, combine the following in a sterile PCR tube:

Total RNA	25.0 ng to 5.0	µg
GE ^{**} (5X gDNA Elimination Buffer)	2.0	µl
H₂O to a final volume of	10.0	µl

****The RT² First Strand Kit (C-03) is not compatible with the chemicals in Ambion's DNA-free™ kits. If your RNA sample has been treated with Ambion's DNA-free™ reagents, please call SABiosciences Technical Support at 1-888-503-3187.**

- b. Mix the contents gently with a pipettor followed by brief centrifugation.
- c. Incubate at 42°C for 5 min.
- d. Chill on ice immediately for at least one minute.

3. Prepare the RT Cocktail:

RT Cocktail	1 reaction	2 reactions	4 reactions
BC3 (5X RT Buffer 3)	4 µl	8 µl	16 µl
P2 (Primer & External Control Mix)	1 µl	2 µl	4 µl
RE3 (RT Enzyme Mix 3)	2 µl	4 µl	8 µl
H₂O	3 µl	6 µl	12 µl
Final Volume	10 µl	20 µl	40 µl

4. First Strand cDNA Synthesis Reaction:

- a. Add 10 µl of RT Cocktail to each 10-µl Genomic DNA Elimination Mixture.
- b. Mix well but gently with a pipettor.
- c. Incubate at 42°C for **exactly** 15 min and then **immediately** stop the reaction by heating at 95°C for 5 minutes.
- d. Add 91 µl of H₂O to each 20-µl of cDNA synthesis reaction. Mix well.
- e. Hold the finished First Strand cDNA Synthesis Reaction on ice until the next step or store overnight at -20°C.

5. RNA Quality Control Check (Optional):

If desired, proceed to characterize a small aliquot (6 µl) of the diluted cDNA template on the correct species-specific and instrument-specific RT² RNA QC PCR Array following the instructions provided in its User Manual. Save the remainder at – 20°C.

C. Performing Real-Time PCR

1. 96-well & 384-well Block Formats

NOTE: The use of SABiosciences's RT² qPCR Master Mixes is critical for obtaining the most accurate results from the PCR Array. Be sure to use the correct master mix for your instrument before continuing with this protocol (See Pages 8 and 9).

NOTE: An incorrectly chosen PCR Array plate format will not properly fit into your real-time PCR instrument, and its use will damage the instrument. Be sure you have the correct PCR Array format for your instrument before continuing with this protocol (See Page 7).

NOTE: The accuracy and precision of your pipetting determine the consistency of your results. Be sure that all of your micro-pipettors are calibrated before beginning this procedure. Also, make sure to not introduce any bubbles into the wells of the PCR Array.

NOTE: If unsure of your RNA quality or isolation technique, examine the quality of your RNA before this step using SABiosciences's species- and instrument-specific RT² RNA QC PCR Arrays (See Page 15).

NOTE: Do not use DEPC treated H₂O. Use high-quality, nuclease-free H₂O. If you are not sure whether your RNase, DNase-free water has been DEPC treated, please check with the supplier.

NOTE: If precipitates are present in the Master Mix tubes, please contact a technical applications scientist at 1-888-503-3187 or support@SABiosciences.com for further instructions.

NOTE: Carefully pipette reagents from reagent tubes, starting with pipette tip at top of tube and working down slowly.

1. Briefly (10-15 seconds) spin down all reagents.
2. **Experimental Cocktail Preparation (for Custom PCR Arrays & Plate H (BioMark) PCR Arrays, see NOTES below):**

Mix the following components in a 5-ml tube or a multi-channel reservoir:

Plate Format: Plate Format Designation:	96-well A, C, D, & F	384-well (4x96) E & G	384HT E & G
2X SABiosciences RT ² qPCR Master Mix	1350 µl	550 µl	2000 µl
Diluted First Strand cDNA Synthesis Reaction	102 µl	102 µl	102 µl
H ₂ O	1248 µl	448 µl	1898 µl
Total Volume	2700 µl	1100 µl	4000 µl

NOTE: This recipe provides an excess volume of ONLY ~ 290 µl (for the 96-well format) to allow for multiple pipetting. Very carefully add the cocktail to the PCR Array precisely as described below to ensure that each well receives the required volume.

NOTE: *If you did not perform RNA quality control with a RT² RNA QC PCR Array, save the remainder of 9 µl of the cDNA synthesis reaction at -20°C in case you need to perform one later for troubleshooting purposes.*

CUSTOM PCR ARRAY NOTE: Prepare Experimental Cocktail for each sample by calculating enough of each component based on the PCR Array layout selected. Please prepare an excess of 10% for pipetting error.

Fluidigm BioMark Format H Plates: Please refer to the RT² cDNA Synthesis Kit – BioMark (Cat #C-10/330431) for instructions on how to set up your plates.

3. Loading the PCR Arrays

Please select your PCR Array Format for loading instructions.

NOTE: *Change pipet tips following each addition to avoid any cross-contamination between the wells or reactions.*

NOTE: *If using robotic instrumentation for loading PCR Arrays, please contact the SABiosciences Technical Support Team for PCR Array plate specifications.*

a. Loading the 96-Well PCR Array Formats A, C, D, or F

- i. **CAREFULLY** remove the PCR Array from its sealed bag.
- ii. **(Optional):** Dispense Experimental Cocktail to RT² PCR Array Loading Reservoir (PA-027/338162) to assist in loading.
- iii. Add 25 µl of the Experimental Cocktail to each well of the PCR Array, preferably from a reservoir with an eight-channel pipettor (or a twelve-channel pipettor but only using eight tips).
- iv. Proceed to the next section (**STEP 3**) on “Performing Real-Time PCR Detection”.

b. Loading the 384-Well PCR Array Formats E or G:

NOTE: *Each 384-well plate characterizes four samples in separate sets of 96-wells staggered from one another by only one well. The spacing between the tips of standard multi-channel pipettors will allow you to properly skip rows or columns when adding each sample. Be sure to load each sample into the correct set of wells. Use Figure 4 as a guide.*

- i. **CAREFULLY** remove the PCR Array from its sealed bag.
- ii. **(Optional):** Dispense Experimental Cocktail to RT² PCR Array Loading Reservoir (PA-027/338162) to assist in loading.
- iii. Load sample cocktails to appropriate wells of the PCR Array, preferably from a reservoir with an eight-channel pipettor (or a twelve-channel pipettor but only using eight tips), using the provided 384EZLoad™ Covers (Catalog #PA-384/338125) and the figure below as a guide.

- Place Cover #1 (white) on the plate. Add 10 μ L of Sample 1 cocktail to the open wells. (Odd number wells of rows A, C, E, G, I, K, M & O). Remove & discard the cover.
- Place Cover #2 (yellow) on the plate. Add 10 μ L of Sample 2 cocktail to the open wells. (Even number wells of rows A, C, E, G, I, K, M & O). Remove & discard the cover.
- Place Cover #3 (black) on the plate. Add 10 μ L of Sample 3 cocktail to the open wells. (Odd number wells of rows B, D, F, H, J, L, N & P). Remove & discard the cover.
- Place Cover #4 (red) on the plate. Add 10 μ L of Sample 4 cocktail to the open wells. (Even number wells of rows B, D, F, H, J, L, N & P). Remove & discard the cover.

Sample #1

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
B	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
D	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
E	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
F	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
H	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
I	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
J	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
K	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
L	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
M	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
N	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
O	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
P	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26

Sample #2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

Sample #3

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3

Sample #4

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	A
B	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	B
C	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	C
D	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	D
E	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	E
F	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	F
G	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	G
H	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	H
I	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	I
J	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	J
K	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	K
L	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	L
M	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	M
N	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	N
O	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	O
P	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	3	4	P
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

Figure 4: To load a 384-well format PCR Array, add 10 μ l of the Experimental Cocktail from each numbered sample into the staggered wells with the same number as indicated in the figure.

- Proceed to the next section (**STEP 3**) on “Performing Real-Time PCR Detection”.
- c. **Loading the PCR Array 384HT**
- CAREFULLY** remove the PCR Array from its sealed bag.
 - (Optional):** Dispense Experimental Cocktail to RT² PCR Array Loading Reservoir (PA-027/338162) to assist in loading.
 - Add 10 μ l of the Experimental Cocktail to each well of the PCR Array, preferably from a reservoir with an 8-channel pipettor (or a 12-channel pipettor but only using 8 tips).
 - Proceed to the next section (**STEP 3**) on “Performing Real-Time PCR Detection”.

d. Loading Custom Format PCR Arrays

- i. **CAREFULLY** remove the PCR Array from its sealed bag.
- ii. **(Optional):** Dispense Experimental Cocktail to RT² PCR Array Loading Reservoir (PA-027/338162) to assist in loading.
- iii. Add each Sample Experimental Cocktail to each well of the PCR Array.
 - i. 25 µl per well for 96-well Custom PCR Arrays
 - ii. 10 µl per well for 384-well Custom PCR Arrays.
- iv. Proceed to the next section (**STEP 4**) on “Performing Real-Time PCR Detection”.

4. Performing Real-Time PCR Detection:

ATTENTION Users of Bio-Rad and Eppendorf Real-Time Instruments: *Prior to initiating the run, please make sure your instrument has been calibrated to use clear, flat optical caps with PCR Array plates.*

NOTE: *Be sure to follow the manufacturer’s instructions for the proper operation and maintenance of your real-time instrument.*

- a. **CAREFULLY** but tightly seal the PCR Array with the optical thin-wall 8-cap strips (Formats A and D) or with the optical adhesive film (Formats C, E, F, and G).
- b. **Centrifuge the plate** for 1 full minute at room temperature at 1000 g to remove bubbles. Visually inspect the plate from underneath of the plate to ensure no bubbles are present in each well.

NOTE: *Bubbles remaining in the bottom of the wells of a PCR Array will interfere with results.*

- c. Place the plate on ice while setting up the PCR cycling program below.
- d. Place one plate in your real-time thermal cycler. If recommended by your instrument’s user manual, use a compression pad with the optical film-sealed plate formats.

NOTE: *PCR Arrays containing experimental cocktail may be store at -20°C wrapped in aluminum foil for up to one week until ready to run.*

- e. Enter and run the appropriate program for your real-time instrument (below). If prompted by your instrument software, select “Absolute Quantitation” to begin.

Use a **two-step cycling program** for the following instrumentation:

ABI: 5700, 7000, 7300, 7500, 7700, 7900HT, StepOnePlus, ViiA 7 Bio-Rad: iCycler, iQ5, MyiQ, MyiQ2, CFX96*, CFX384* Eppendorf*: Mastercycler ep realplex 2, 2S, 4, 4S Stratagene: Mx3000p, Mx3005p, Mx4000p	Cycles	Duration	Temperature
	1	10 minutes ¹	95°C
	40	15 seconds	95°C
		1 minute ²	60°C

***Attention** Bio-Rad CFX96 & CFX384 Users:

- **Adjust the ramp rate to 1°C/sec.**

****Attention** Eppendorf Mastercycler ep realplex 2, 2S, 4, and 4S Users:

- **For the Silver Thermoblock: Adjust the ramp rate to 26%.**
- **For the Aluminum Thermoblock: Adjust the ramp rate to 35%.**
- Please refer to the Instrument Setup Guide at <http://sabiosciences.com/pcrarrayprotocolfiles.php> for detailed setup instructions.

Use an **extended two-step cycling program** for the following instrumentation:

Roche***: LightCycler 480	Cycles	Duration	Temperature
	1	10 minutes ¹	95°C
	45	15 seconds	95°C
		1 minute ²	60°C

*****Attention** Roche LightCycler 480 Users: **Adjust the ramp rate to 1°C/sec.** Please refer to the Instrument Setup Guide at <http://sabiosciences.com/pcrarrayprotocolfiles.php> for more information on other **REQUIRED** changes to settings for Melt Curve Acquisition.

Use a **three-step cycling program** for the following instrumentation:

Bio-Rad: Opticon, Opticon 2, Chromo 4 (MJ Research) Takara: TP-800 All other instruments	Cycles	Duration	Temperature
	1	10 minutes ¹	95°C
	40	15 seconds	95°C
		30 to 40 seconds ^{2,3}	55°C
		30 seconds	72°C

¹ The 10-minute step at 95°C is required to activate the HotStart DNA polymerase.

² Detect and record SYBR Green fluorescence from every well during the annealing step of each cycle.

³ Different instruments need different lengths of time to detect the fluorescent signal. Choose the appropriate time for the annealing step (55°C) for your instrument.

NOTE: For additional help with instrument setup, see our Instrument-Specific Setup Instructions and Protocol Files at: www.SABiosciences.com/pcrarrayprotocolfiles.php

- e. Calculate the threshold cycle (C_t) for each well using the instrument's software.

NOTE: For Roche LightCycler 480 Users, there are two options available to analyze your data.

1. Use the second derivate max setting & there is no need to set a threshold, **OR**
 2. Use "Fit Points" and follow instruction ii below.
- i. **To define the Baseline**, choose the Automated Baseline option if your instrument has the Adaptive Baseline Function (check with instrument manual or manufacturer, if unsure). If it does not have the adaptive baseline function, you will need to set the baseline manually. Use the Linear View of the amplification plots to determine the earliest visible amplification. Set the instrument to use the readings from cycle number two (2) through two (2) cycles before the earliest visible amplification, but no more than cycle 15. The earliest amplifications usually will be visible between cycles 14 and 18.
- ii. **Manually define the Threshold Value** by using the Log View of the amplification plots and place it above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.

***IMPORTANT:** Ensure that the thresholds are the same across all PCR Array runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across arrays. If the RNA sample quality has been adequately controlled, the cycling program has been executed properly, and the thresholds have been defined correctly, then the value of C_t^{PPC} should be 20 ± 2 across all of your arrays or samples. If not, see the Troubleshooting and FAQ section.

- iii. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for use with the SABiosciences PCR Array Data Analysis Template Excel or Web-based Utilities.
- ii. Excel-based PCR Array Data Analysis Templates available for 96-well, 384-well (4x96), 384HT, and Custom PCR Array formats available at:
 1. <http://sabiosciences.com/pcrarraydataanalysis.php>
 - iii. Web-based PCR Array Data Analysis Software at:
 1. <http://sabiosciences.com/pcrarraydataanalysis.php>

5. Recommended Quality Control: Dissociation (Melting) Curve

For instrument-specific melt curve analysis settings, please refer to the corresponding Instrument Setup Guide for your instrument at:

<http://sabiosciences.com/pcrarrayprotocolfiles.php>

NOTE: If you decide not to obtain the dissociation curve immediately, save the plates wrapped in aluminum foil at -20°C as is, in case you need to perform this operation at a later point in time for troubleshooting purposes. When ready, simply warm the plate to room temperature, place it into your real-time instrument, and run the melting program described above.

NOTE: *Be sure to visually inspect the plate after the run for any signs of evaporation from any of the wells. If evaporation is observed, make a note of which wells so that you may qualify your data analysis appropriately.*

NOTE: *DO NOT open any previously run and stored PCR Array plate. Removing the thin-wall 8-cap strips or the adhesive film from PCR Arrays releases PCR product DNA into the air where it will contaminate and confound the results of future real-time PCR experiments. See also the Note on “Preparing a Workspace Free of DNA Contamination”.*

General Protocol:

Run a melting curve program immediately after the above cycling program, and generate a first derivative dissociation curve for each well in the entire plate using your instrument's software. No more than one peak should appear in each reaction at temperatures greater than 80°C. If your instrument does not have a default melting curve program, run the following program instead:

95°C, 1 min; 65°C, 2 min (OPTICS OFF); 65°C to 95°C at 2°C / min (OPTICS ON).

2. 100-well “R” Format PCR Array on Rotor-Disc 100 for Rotor-Gene Q

NOTE: The use of SABiosciences’s RT² FAST qPCR Master Mixes is critical for obtaining the most accurate results from the PCR Array. Be sure to use the correct master mix for your instrument before continuing with this protocol (See Pages 8 and 9).

NOTE: The accuracy and precision of your pipetting determine the consistency of your results. Be sure that all of your micro-pipettors are calibrated before beginning this procedure. Also, make sure to not introduce any bubbles into the wells of the PCR Array.

NOTE: If unsure of your RNA quality or isolation technique, examine the quality of your RNA before this step using SABiosciences’s species- and instrument-specific RT² RNA QC PCR Arrays (See Page 15).

NOTE: Do not use DEPC treated H₂O. Use high-quality, nuclease-free H₂O. If you are not sure whether your RNase, DNase-free water has been DEPC treated, please check with the supplier.

NOTE: If precipitates are present in the Master Mix tubes, please contact a technical applications scientist at 1-888-503-3187 or support@SABiosciences.com for further instructions.

NOTE: Carefully pipette reagents from reagent tubes, starting with pipette tip at top of tube and working down slowly.

1. Briefly (10-15 seconds) spin down all reagents.
2. **Experimental Cocktail Preparation**

Mix the following components in a 5-ml tube:

Array Format: Array Format Designation:	100-well R
2X SABiosciences RT ² Fast SYBR Green/Rox PCR Master Mix	1150 µl
Diluted First Strand cDNA Synthesis Reaction	102 µl
H ₂ O	1048 µl
Total Volume	2300 µl

NOTE: This recipe provides an excess volume of ONLY ~300 µl (for the 100-well R format). Very carefully add the cocktail to the PCR Array precisely as described below to ensure that each well receives the required volume.

NOTE: If you did not perform RNA quality control with a RT² RNA QC PCR Array, save the remainder of 9 µl of the cDNA synthesis reaction at -20°C in case you need to perform one later for troubleshooting purposes.

3. Loading the Rotor-Gene Q Rotor-Disc 100 PCR Array

NOTE: *Change pipet tips following each addition to avoid any cross-contamination between the wells or reactions.*

- a. **CAREFULLY** remove the PCR Array from its sealed bag and slide the array into the Rotor-Disc 100 loading block using the tab at position A1 and the tube guide holes.
- b. Manually dispense 20 µl of the Experimental Cocktail into wells 1 through 100 of the PCR Array (although wells 97-100 do not contain assays, their loading is essential for optimized balancing of the Rotor-Gene Q 100 PCR array). Alternatively, manual additional of the Experimental Cocktail can be avoided by using the QIAgility, a compact benchtop instrument that provides rapid, high-precision PCR setup (*please contact a technical applications scientist at 1-888-503-3187 or support@SABiosciences.com for further information*).
- c. Proceed to the next section (**STEP 4**) on “Performing Real-Time PCR Detection using the Rotor-Gene Q”.

4. Performing Real-Time PCR Detection using the Rotor-Gene Q:

NOTE: *Be sure to follow the manufacturer’s instructions for the proper operation and maintenance of your real-time instrument.*

- f. **CAREFULLY** seal the Rotor-Gene Q 100 PCR Array with the Rotor-Disc heat sealing film using the Rotor-Disc heat sealer. Allow one minute to pass, and remove excess sealing film.
- g. Insert Rotor-Gene Q 100 PCR Array into Rotor-Disc 100 rotor. Subsequently attach the Rotor-Disc 100 locking ring.
- h. Insert loaded rotor with attached locking ring into Rotor-Gene Q real-time PCR machine.

NOTE: *PCR Arrays containing experimental cocktail may be store at -20°C wrapped in aluminum foil for up to one week until ready to run.*

- i. Enter and run the appropriate program (below).

QIAGEN: Rotor-Gene Q 100	Cycles	Duration	Temperature
	1	10 minutes ¹	95°C
	40	10 seconds 30 seconds ²	95°C 60°C

¹ The 10-minute step at 95°C is required to activate the HotStart DNA polymerase.

² Detect and record SYBR Green fluorescence from every well during the annealing step of each cycle.

NOTE: *For additional help with instrument setup, see our Instrument-Specific Setup Instructions and Protocol Files at: www.SABiosciences.com/pcrarrayprotocolfiles.php*

- f. Calculate the threshold cycle (C_t) for each well using the instrument's software.

***NOTE:** For more details on data analysis parameters specific for the Rotor-Gene Q, please consult the Rotor-Gene Q Manual.

iv. **To define the Baseline:**

- a. Select **Dynamic Tube** (default analysis setting) to ensure the average background of each well is determined just before amplification commences.
- b. Optional) Select **Ignore First**. Fluorescent signal from the initial cycles may not be representative of the remainder of the run. Thus, better results may be achieved if the initial cycles are ignored. Up to 5 cycles can be ignored.
- c. (Optional) Select **Noise Slope Correction**. Selection of this option can improve data whose baseline (initial cycles) is noticeably sloped. Noise Slope Correction improves the data when raw data backgrounds are observed to slope upward or downward before the takeoff point (C_t).

***IMPORTANT:** Ensure that all selections remain consistent across all PCR Array runs in the same analysis.

- v. **Manually define the Threshold Value** by using the Log View of the amplification plots and place it above the background signal but within the lower one-third to lower one-half of the linear phase of the amplification plot.

***IMPORTANT:** Ensure that the thresholds are the same across all PCR Array runs in the same analysis. The absolute position of the threshold is less critical than its consistent position across arrays. If the RNA sample quality has been adequately controlled, the cycling program has been executed properly, and the thresholds have been defined correctly, then the value of C_t^{PPC} should be 14 ± 2 across all of your arrays or samples. If not, see the Troubleshooting and FAQ section.

- vi. Export the resulting threshold cycle values for all wells to a blank Excel spreadsheet for use with the SABiosciences PCR Array Data Analysis Template Excel or Web-based Utilities.

- i. Excel-based PCR Array Data Analysis Templates available for 96-well, 384-well (4x96), 384HT, Rotor-Gene Q, and Custom PCR Array formats available at:

1. <http://sabiosciences.com/pcrarraydataanalysis.php>

- ii. Web-based PCR Array Data Analysis Software at:

1. <http://sabiosciences.com/pcrarraydataanalysis.php>

5. Recommended Quality Control: Dissociation (Melting) Curve

For Rotor-Gene Q melt curve analysis settings, please refer to the corresponding Instrument Setup Guide for your instrument at:

<http://sabiosciences.com/pcrarrayprotocolfiles.php>

NOTE: *Be sure to visually inspect the Rotor-Gene Q Ring after the run for any signs of evaporation from any of the wells. If evaporation is observed, make a note of which wells so that you may qualify your data analysis appropriately.*

NOTE: *DO NOT open any previously run and stored PCR Array rings. Removing the adhesive film from PCR Arrays releases PCR product DNA into the air where it will contaminate and confound the results of future real-time PCR experiments. See also the Note on “Preparing a Workspace Free of DNA Contamination”.*

General Protocol:

Run a melting curve program immediately after the above cycling program, and generate a first derivative dissociation curve for each well in the entire plate using your instrument's software. Melt curve analysis can be added during creation of the Rotor-Gene Q qPCR program. No more than one peak should appear in each reaction.

D. Data Analysis: $\Delta\Delta C_t$ Method

1. For 96-well & 384-well Formats

PCR Array Data Analysis Web Portal (Excel & Web-based Utilities)

Access our free PCR Array Data Analysis Web Portal from the following address:

<http://www.SABiosciences.com/pcrarraydataanalysis.php>

The PCR Array Data Analysis Web Portal automatically performs the following calculations and interpretation of the control wells upon including threshold cycle data from a real-time instrument. The PCR Array Data Analysis Web Portal presents the results in a tabular format, a scatter plot, a three-dimensional profile, and a volcano plot (when replicates are included).

1. Change all C_t values reported as greater than 35 or as N/A (not detected) to 35. At this point, any C_t value equal to 35 is considered a negative call.
2. Examine the threshold cycle values of the control wells.
 - a. Genomic DNA Control (GDC):
 - i. Calculate C_t^{GDC} .
 - ii. If the value is greater than 35, then the level of genomic DNA contamination is too low to affect gene expression profiling results. No action is needed.
 - iii. If the value is less than 35, then genomic DNA contamination is evident. See the Troubleshooting and FAQ section.
 - b. Reverse Transcription Control (RTC):

Any impurities in your RNA sample that affect the reverse transcription of the RT² First Strand Kit's built-in external RNA control also affect the reverse transcription of your messages of interest.

 - i. Calculate $\Delta C_t = \text{AVG } C_t^{RTC} - \text{AVG } C_t^{PPC}$.
 - ii. If this value is less than 5, then no inhibition is apparent.
 - iii. If this value is greater than 5, then evidence of impurities that inhibited the reverse transcription phase of the procedure is evident. See the Troubleshooting and FAQ section.

NOTE: For RT² Nano PreAMP and RT² FFPE PreAMP Users

Please refer to corresponding user manuals for correct settings and GDC, PPC, and RTC values.

c. Positive PCR Control (PPC):

Any impurities in your RNA sample that affect the PCR amplification of the positive control also affect the PCR amplification for your messages of interest.

- i. The average C_t^{PPC} value should be 20 ± 2 on each PCR Array and should not vary by more than two cycles between PCR Arrays being compared.
- ii. Larger differences in average C_t^{PPC} values between samples indicate the presence of different amounts of PCR amplification inhibitors in each sample and that all of the RNA samples require further purification.
- iii. An average value of C_t^{PPC} that is consistently greater than 22 for all of your samples may indicate a problem with the cycling conditions or may simply be indicative of the relative sensitivity of your instrument. See the Troubleshooting and FAQ section.

3. Calculate the ΔC_t for each pathway-focused gene in each plate.

$$\Delta C_t = C_t^{GOI} - C_t^{AVG\ HKG}$$

NOTE: Choosing the right normalization factor

The expression level of the housekeeping genes chosen for normalization in the $\Delta\Delta C_t$ method must not be influenced by your experimental conditions. If one or more such genes have been previously identified by independent means and if the PCR Array reproduces those results, use the average of their C_t values in the equation above. If an appropriate housekeeping gene has not been previously identified, use the average C_t value of all housekeeping genes. Or, simply use zero (0) in the place of the average of HK genes' C_t for each group to be compared, and rely on the consistency in the quantity and quality of your original input total RNA across your groups to effectively normalize your results.

4. When biological and/or technical replicates are performed, calculate the average ΔC_t value of each gene (each well) across those replicate arrays for each treatment group.
5. Calculate the $\Delta\Delta C_t$ for each gene across two PCR Arrays (or groups).

$$\Delta\Delta C_t = \Delta C_t (\text{group 2}) - \Delta C_t (\text{group 1})$$
 Where group 1 is the control and group 2 is the experimental
6. Calculate the fold-change for each gene from group 1 to group 2 as $2^{-\Delta\Delta C_t}$.

OPTIONAL: *If the fold-change is greater than 1, then the result may be reported as a fold up-regulation. If the fold-change is less than 1, then the negative inverse of the result may be reported as a fold down-regulation. The fold-change ratios may also be reported as is.*

(Optional) Go From Fold Change to Biology in Minutes with the New GeneWeb Tool

– Go to <http://geneweb.sabiosciences.com>

2. For 100-well “R” Format PCR Array on Rotor-Disc 100 for Rotor-Gene Q

PCR Array Data Analysis Web Portal (Excel & Web-based Utilities)

Access our free PCR Array Data Analysis Web Portal from the following address:

<http://www.SABiosciences.com/pcrarraydataanalysis.php>

The PCR Array Data Analysis Web Portal automatically performs the following calculations and interpretation of the control wells upon including threshold cycle data from a real-time instrument. The PCR Array Data Analysis Web Portal presents the results in a tabular format, a scatter plot, a three-dimensional profile, and a volcano plot (when replicates are included).

1. Change all C_t values reported as greater than 33 or as N/A (not detected) to 33. At this point, any C_t value equal to 33 is considered a negative call.
2. Examine the threshold cycle values of the control wells.
 - d. Genomic DNA Control (GDC):
 - i. Calculate C_t^{GDC} .
 - ii. If the value is greater than 33, then the level of genomic DNA contamination is too low to affect gene expression profiling results. No action is needed.
 - iii. If the value is less than 33, then genomic DNA contamination is evident. See the Troubleshooting and FAQ section.
 - e. Reverse Transcription Control (RTC):

Any impurities in your RNA sample that affect the reverse transcription of the RT² First Strand Kit's built-in external RNA control also affect the reverse transcription of your messages of interest.

 - i. Calculate $\Delta C_t = \text{AVG } C_t^{RTC} - \text{AVG } C_t^{PPC}$.
 - ii. If this value is less than 5, then no inhibition is apparent.
 - iv. If this value is greater than 5, then evidence of impurities that inhibited the reverse transcription phase of the procedure is evident. See the Troubleshooting and FAQ section.

f. Positive PCR Control (PPC):

Any impurities in your RNA sample that affect the PCR amplification of the positive control also affect the PCR amplification for your messages of interest.

- i. The average C_t^{PPC} value should be 14 ± 2 on each PCR Array and should not vary by more than two cycles between PCR Arrays being compared.
- ii. Larger differences in average C_t^{PPC} values between samples indicate the presence of different amounts of PCR amplification inhibitors in each sample and that all of the RNA samples require further purification.
- iii. An average value of C_t^{PPC} that is consistently greater than 16 for all of your samples may indicate a problem with the cycling conditions or may simply be indicative of the relative sensitivity of your instrument. See the Troubleshooting and FAQ section.

3. Calculate the ΔC_t for each pathway-focused gene in each plate.

$$\Delta C_t = C_t^{GOI} - C_t^{AVG\ HKG}$$

NOTE: Choosing the right normalization factor

The expression level of the housekeeping genes chosen for normalization in the $\Delta\Delta C_t$ method must not be influenced by your experimental conditions. If one or more such genes have been previously identified by independent means and if the PCR Array reproduces those results, use the average of their C_t values in the equation above. If an appropriate housekeeping gene has not been previously identified, use the average C_t value of all housekeeping genes. Or, simply use zero (0) in the place of the average of HK genes' C_t for each group to be compared, and rely on the consistency in the quantity and quality of your original input total RNA across your groups to effectively normalize your results.

4. When biological and/or technical replicates are performed, calculate the average ΔC_t value of each gene (each well) across those replicate arrays for each treatment group.
5. Calculate the $\Delta\Delta C_t$ for each gene across two PCR Arrays (or groups).
 $\Delta\Delta C_t = \Delta C_t (\text{group 2}) - \Delta C_t (\text{group 1})$
 Where group 1 is the control and group 2 is the experimental
6. Calculate the fold-change for each gene from group 1 to group 2 as $2^{(-\Delta\Delta C_t)}$.

NOTE: Detailed Mathematical Explanation of $\Delta\Delta C_t$ Data Analysis Method

Due to the inverse proportional relationship between the threshold cycle (C_t) and the original gene expression level, and the doubling of the amount of product with every cycle, the original expression level (L) for each gene of interest is expressed as:

$$L = 2^{-C_t}$$

To normalize the expression level of a gene of interest (GOI) to a housekeeping gene (HKG), the expression levels of the two genes are divided:

$$\frac{2^{-C_t(\text{GOI})}}{2^{-C_t(\text{HKG})}} = 2^{-[C_t(\text{GOI}) - C_t(\text{HKG})]} = 2^{-\Delta C_t}$$

To determine fold change in gene expression, the normalized expression of the GOI in the experimental sample is divided by the normalized expression of the same GOI in the control sample:

$$\frac{2^{-\Delta C_t(\text{expt})}}{2^{-\Delta C_t(\text{control})}} = 2^{-\Delta\Delta C_t} \quad \text{Where } \Delta\Delta C_t \text{ is equal to } \Delta C_t(\text{expt}) - \Delta C_t(\text{control})$$

The complete calculation is as follows:

$$\frac{\frac{2^{-\Delta C_t(\text{GOI})} \text{ expt}}{2^{-\Delta C_t(\text{HKG})} \text{ expt}}}{\frac{2^{-\Delta C_t(\text{GOI})} \text{ control}}{2^{-\Delta C_t(\text{HKG})} \text{ control}}} = \frac{2^{-[C_t(\text{GOI}) - C_t(\text{HKG})]} \text{ expt}}{2^{-[C_t(\text{GOI}) - C_t(\text{HKG})]} \text{ control}} = \frac{2^{-\Delta C_t} \text{ expt}}{2^{-\Delta C_t} \text{ control}} = 2^{-\Delta\Delta C_t}$$

VI. Troubleshooting and FAQs

A. Troubleshooting:

1. Removal of Genomic DNA Contamination:

You must perform the on-column DNase treatment step included in the protocol of Qiagen's RNeasy Mini Kit (Catalog # 74104). You must also then use SABiosciences's RT² First Strand Kit (C-03/330401) with its genomic DNA elimination step.

If the genomic DNA contamination proves difficult to remove, fold-changes in gene expression may still be obtained. However, it will then be very important to validate any results for individual genes by a separate more rigorous real-time PCR analysis that includes a "minus RT" control.

Apparent genomic DNA contamination may also indicate evidence of more general DNA contamination of other reagents, tips, and tubes. See the Note about Preparing a Workspace Free of DNA Contamination at the beginning of the protocol in this User Manual. The No Template Control (NTC) in the RT² RNA QC PCR Array provides a sense of how well your technique minimizes the introduction of general DNA contamination into your assay system.

2. Improving Poor Reverse Transcription Efficiency:

Double-check the A260:A280 and A260:A230 ratios of your RNA samples and be sure to perform the dilutions for spectrophotometry in RNase-free Tris pH 8.0 buffer. If necessary, re-purify your RNA samples with a spin-column based clean up method, such as Qiagen's RNeasy Mini Kit (Catalog # 74104), with the subsequent RNase-Free DNase Set: 79254.

3. Improving Poor PCR Amplification Efficiency:

Different instruments have different levels of sensitivity. If an average C_t^{PPC} value of 20 ± 2 is difficult to obtain for your instrument, the observed average C_t^{PPC} value should be acceptable as long as it does not vary by more than two cycles between PCR Arrays being compared.

Be sure that the initial heat activation step at 95°C had been lengthened to 10 minutes from the shorter time in the default program. Be sure that all other cycle parameters also have been correctly entered according to the recommendations in this User Manual. Also, double check the quality of your RNA as described in "Evidence of Poor Reverse Transcription Efficiency" above.

B. Frequently Asked Questions:

1. Will pipetting error affect the PCR Array results?

The passive reference dyes in the master mixes, such as ROX and Fluorescein, are used by the real-time PCR systems to normalize variation from well to well. Therefore, these systems tolerate volume variations caused by pipetting error and evaporation.

2. How can I prevent the evaporation of reaction volume from the wells?

Be sure to carefully and completely seal the PCR Array with the optical thin-wall 8-cap strips or the optical adhesive film before placing it into your thermal cycler. Also, be sure to use a compression pad with the plate formats using the optical film seal (Formats C, E, F and G) as directed by the manufacturer of your real-time PCR instrument.

3. How reliable are the results from the RT² Profiler PCR Array?

Assuming the use of good, consistent experimental technique, real-time PCR methods such as the PCR Array provide very reproducible results. To insure the reliability of your results and to reliably detect smaller fold changes in gene expression from the PCR Array, the performance of replicate determinations (such as biological triplicates) is highly recommended. The Data Analysis Template available from our website for the PCR Array uses your replicate PCR Array data to calculate t-test p values and to generate a “Volcano Plot” illustrating the statistically significant fold-changes in gene expression.

4. Can I use cDNA synthesized from a first-strand kit from another manufacturer?

If your cDNA template is not synthesized using an SABiosciences RT² First Strand Kit, cDNA from 500 ng to 5 µg total RNA may be used.

If the cDNA synthesis reaction volume was:

- 20 µl or more, add H₂O to the synthesized cDNA to increase the volume to 111 µl, and proceed as directed in Section B, Step 4e.
- less than 20 µl, add H₂O to the synthesized cDNA to increase the volume to 20 µl and proceed as directed in Section B, Step 4d.

5. Do I need to prepare my reactions or pre-mix on ice?

You can prepare your reactions at room temperature. Since the SABiosciences RT² Master Mixes include a Hot-Start DNA Polymerase that is active only upon heat activation, you can be assured that no non-specific amplification results will be produced.

If you have additional questions, please check our website (www.SABiosciences.com) for a more complete listing of Frequently Asked Questions (FAQs), or call our Technical Support Representatives at 1-888-503-3187 or 301-682-9200.

Technical Support: support@SABiosciences.com www.SABiosciences.com

Appendix: Modified Protocol for Housekeeping Gene PCR Arrays

A. First Strand cDNA Synthesis

Perform a first strand cDNA synthesis reaction for each sample to be characterized on the array including one sample representing your biological or experimental control.

B. Perform Real-Time PCR:

1. Experimental Cocktail Preparation:

Mix the following components in a 1-ml tube or a multi-channel reservoir:	
2X SABiosciences RT ² qPCR Master Mix	337.5 μ l
Diluted first strand cDNA synthesis reaction	27 μ l
H ₂ O	310.5 μ l
Total volume	675 μ l

2. Adding samples to PCR Array:

NOTE: Organize your sample loading onto the arrays very carefully making sure to characterize each sample in duplicate and to include a replicate of the control sample on each plate. For example, up to four samples can be characterized in duplicate on a single array or duplicate determinations may be made on two separate arrays for larger numbers of samples.

		Housekeeping Genes											
		1	2	3	4	5	6	7	8	9	10	11	12
Samples	A	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	B	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	C	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	D	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	E	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	F	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
	H	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12

Figure 5: Layout of the Housekeeping Genes PCR Arrays.

C. Data Analysis by the ΔC_t Method

- For each sample, average the duplicate determinations of the C_t values from each sample for each housekeeping gene.
- For each housekeeping gene, calculate the ΔC_t , or, in other words, the difference between the gene's C_t value in each experimental sample and the same gene's C_t value in the control sample.

3. Choose the housekeeping genes with the smallest ΔC_t value across the samples of interest to normalize the results of your future RT-PCR experiments for input total RNA loading. More than one housekeeping gene may be chosen for your analyses. Simply monitor the expression of all of these housekeeping genes, and use their average C_t value as the normalization factor for each sample.

RT² Profiler™ PCR Arrays

NOTICE TO PURCHASERS

- This product is intended for research purposes only and is not intended for drug or diagnostic purposes or for human use. Use of kit components for reproduction of any primer pair mix, to modify kit components for resale or to use RT² Profiler PCR Arrays to manufacture commercial products without written approval of SABiosciences Corporation is expressly prohibited.

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- This warranty limits our liability to the replacement of this product in the event the product fails to perform due to any manufacturing defect. SABiosciences Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose. SABiosciences Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the use, the results of use or the inability to use this product.

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- Patent Pending.

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NOTES

NOTES

RT² Profiler™ PCR Array User Manual

Part #1022A

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